REMARKS

The Official Action dated June 13, 2002 and the references cited therein have been reviewed. Claims 4 to 18 and 37 were pending. Claims 4-7, 11-15, 18 and 37 are amended herein. Corrected drawings will be forwarded to the draftsperson in a separate paper. In view of the foregoing amendments and the following arguments, Applicants respectfully request favorable reconsideration and allowance of all claims under examination.

The present invention is directed to methods of preparing a substantially RNA-free cellular component comprising culturing cells producing a cellular component and cells producing an RNase; lysing the cultured cells to produce a combined cell lysate wherein the cells producing the RNase produce sufficient RNase to degrade substantially all of the RNA present in the combined cell lysate; incubating the combined cell lysate to allow the RNase to digest substantially all the RNA present and isolating the cellular component. It is further directed to methods of preparing a substantially RNA-free cellular component comprising culturing cells producing a cellular component and an RNase, lysing the cultured cells to produce a cell lysate wherein the lysate contains sufficient RNase to degrade substantially all of the RNA present in the lysate; incubating the lysate to allow the RNase to digest substantially all the RNA present and isolating the cellular component, thereby producing a substantially RNA-free cellular component.

In the Office Action dated June 13, 2002, the following were made of record.

1. The examiner stated that claims 4-18 and 37 are pending.

2. The examiner noted that the examination has been assigned to a different examiner within Group Art Unit 1652.

3. The examiner acknowledged the claims for domestic priority under 35 U.S.C. §119(e).

4. The examiner acknowledge the claim for foreign priority and receipt of certified copies of the priority documents.

5. On form PTO-892 the examiner cited the following nonpatent document: Zhu et al. J. Bacteriol. 172(6):3146-3151, 1990.

6. The examiner acknowledged the amendment of claims 4-9 and 11; the addition of claim 37, and the cancellation of claims 1-3 and 36 in Paper No. 17 filed April 4, 2002.

7. The examiner stated that the amendments and/ cancellations from Paper 17 were deemed sufficient to overcome some of the previous rejections, and that rejections and/or objection not reiterated from previous actions are withdrawn.

8. The examiner noted the use of trademarks in the specification and further noted that their status as trademarks should be indicated, as by capitalization.

9. The examiner indicated that drawings have been reviewed and were objected to, that the Notice of Draftsperson's Patent Drawing Review was attached and that corrected drawings were required. The examiner requested that any amendments to the specification necessitated by the drawing corrections be submitted during prosecution.

10. Claim 18 was objected to because the examiner noted that for clarity the expression "said RNase being" should be replaced with "wherein said RNase is."

11. Claims 4-18 and 37 were rejected under 35 U.S.C. §112, second paragraph as allegedly indefinite for failing to particularly point out and distinctly claim the subject matter of the invention.

A) Claims 4 and 37 (and claims 5-18 dependent thereon) were allegedly indefinite for reciting "RNase in an amount sufficient to degrade substantially all of the RNA" because the term "substantially" was deemed a relative term.

B) Claim 4 (and claims 5-18 dependent thereon) was allegedly indefinite for reciting "incubating said lysate" The examiner stated that it is unclear which lysate is being incubated.

C) Claim 7 was allegedly indefinite because the recited term "recombinant carbohydrate" is unclear.

D) Claims 11-15 (and claims 16-18 dependent thereon) were deemed indefinite for the recitation of "regulated manner" which the examiner alleged was unclear.

E) Claims 9 and 18 were allegedly indefinite in the recitation of "non-specific RNase," which the examiner believed to be unclear.

12. Claims 4-18 and 37 were rejected under 35 U.S.C. §112, first paragraph as containing subject matter which was allegedly not described in the specification so as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. Claims 4-18 and 37 further were rejected under 35 U.S.C. §112, first paragraph because the specification, while enabling for a method of preparing RNA-free cellular components in an *E. coli* cell, wherein the cellular component and RNaseI or RNaseA are produced by said *E. coli* and wherein the RNaseI or RNaseA are expressed and secreted using an inducible or constitutive promoter, is allegedly not enabling for a method for preparing RNA-free cellular components wherein any RNase is expressed in any cell and wherein any RNase is expressed constitutively in the cytoplasm.

13. Claims 4, 6-7, 9-13 and 15-18 were rejected under 35 U.S.C. §103(a), as allegedly unpatentable over McMaster et al. (Anal. Biochem. 109:47-54, 1980) in view of Okorokov et al. (Protein Expression and Purification 6:472-480, 1995).

14. Claims 8 and 14 were rejected under 35 U.S.C. §103(a) as being allegedly unpatentable over McMaster et al. in view of Okorokov et al., as above and further in view of Zhu et al. (J. Bact. 172:3146-3151, 1990).

Preliminarily, the specification has been amended to correct typographical errors relating to trademarks. Claims 4-7, 11-15, 18 and 37, have been amended to provide clarity. Support can be found in the specification for any amendments to existing claims. Applicants' undersigned representative states that the amendments to the specification and the claims add no new matter.

Examiner's Objections

The informality relating to the expression "said RNase being" in claim 18 has been corrected. The applicants thank the examiner, and request that the objection be withdrawn.

Rejections under 35 U.S.C. §112, second paragraph

A) Claims 4 and 37 (and claims 5-18 dependent thereon) were allegedly indefinite for reciting "RNase in an amount sufficient to degrade substantially all of the RNA" because the term "substantially" was deemed a relative term. A claim is indefinite merely for reciting a relative term. When a term of degree is presented in a claim, first a determination is to be made as to whether the specification provides some standard for measuring that degree. *MPEP* 2173.05(b). Where the specification contains at least general guidelines for determining the meaning, the courts have held that the term "substantially" is definite. *See In re Nehrenberg,* 280 F.2d 161,126 (CCPA 1960). Here, the specification defines in detail the definition in terms of the amount of residual RNA permissible. Applicants give specific guidance to the meaning of the term "substantially RNA free. "'Substantially RNA-free DNA' refers to the presence in a sample containing the cellular component of less than 1%, preferably less than 0.2% and most preferably less than 0.1% -0.01% (w/w of RNA/DNA in the sample) (application as filed, page 5, lines 1-16). In addition, the working examples provide quantitative measurements reflecting the amount of RNA degraded under experimental conditions. See, for example, application as filed, page 59, lines 4-7. In view of the guidance provided in the specification with respect to the term "substantially", it would be clear to one of skill in the art what is meant by the expression "to degrade substantially all of the RNA." Accordingly, the applicants respectfully request withdrawal of the rejection as to these claims.

B) Claim 4 (and claims 5-18 dependent thereon) was deemed allegedly indefinite for reciting "incubating said lysate" The Office Action alleged that it is unclear which lysate is being incubated. In the claims as amended the recitation "incubating said lysate" has been amended to more fully reflect which cell lysate is being incubated. In view of this amendment, the applicants respectfully request that the rejection be withdrawn.

C) Claim 7 was deemed allegedly indefinite because the recited term "recombinant carbohydrate" is unclear. Applicants have clarified the term in accordance with the examiner's suggestion. In view of the amended claim language, the applicants respectfully request that the rejection be withdrawn.

D) Claims 11-15 (and claims 16-18 dependent thereon) were deemed allegedly indefinite for the recitation of "regulated manner" which the Office Action alleged was unclear. The expression "regulated manner" is clearly defined on page 6 of the specification as follows: '"Regulated manner" refers to gene expression, for example, of an RNase gene in a host cell that is transcriptionally regulated, for example, constitutively or inducibly. It also refers to regulation at the level of protein production, for example, the use of a signal sequence or a fusion protein to direct a protein to the host cell periplasm or out of the host cell and into the medium surrounding the host cell.' (application as filed, page 6, lines 5-9). Notwithstanding this complete definition, the claims have been amended to clarify. The Applicants respectfully request that the rejection be withdrawn.

E) Claims 9 and 18 were deemed allegedly indefinite in the recitation of "non-specific RNase," which the Office Action asserted to be unclear. The use of the term "non-specific" is a term of art which is understood by those of skill in the art most connected with the use of RNases to mean a non base specific RNase. A suitable definition of the term is found on page ten of the specification as filed, at lines 14 through 18. "Non-specific RNases hydrolyze phosphodiester bonds in single stranded or double stranded RNA or RNA/DNA molecules, anywhere along the length of the RNA molecule." Further, art cited by the examiner (Zhu et al.,*J. Bact. 172:3146, 1990*) uses the term "nonspecific" referring to the recognition properties of the RNase enzyme in at least two places – for example see page 3146, column 1, paragraph 1, and page 3149, column 2, paragraph 5, wherein, for example, it is stated "This result was surprising, since RNase I is a very active, nonspecific nuclease and would have been expected to degrade much of the cellular RNA." When viewed in context of the art, and the instant specification as a whole, it is clear to those of skill in the art what is meant by "non-specific" with respect to RNase activity. *SeeMPEP* 2173.05(a). The applicants respectfully request that the rejection be withdrawn.

Rejections under 35 U.S.C. §112, first paragraph

Claims 4-18 and 37 were rejected under 35 U.S.C. §112, first paragraphas allegedly not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention at the time of filing.

The written description requirement of section 112 is set forth as follows:

> "The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person of skill in the art to which it pertains or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention."
>
> 35 U.S.C. §112, first paragraph (2002).

The claims have been amended to more fully clarify the nature of the cells producing the RNase.

The Office Action concedes that the specification teaches the use of *E. coli* for the expression of RNases I and A, as well as *E. coli*capable of expressing a cellular component wherein the *E. coli* further expresses and secretes RNase I and/or RNase A using inducible or constitutive promoters. The Office Action further concedes that the use of *S. typhimurium*, *Bacillus*, *Streptomyces* and *Pseudomonas* are taught. The Office Action alleges, however, that no disclosure of other host cells or RNases is described. In addition, the Office Action states that some knowledge or guidance as to which promoters can be used in different hosts is required to practice the claimed method.

In addition to the disclosure acknowledged by the examiner, the instant specification provides further teachings and guidance as shown below.

Despite the contention in the Office Action that no disclosure of other RNases is present, at pages 10 and 11 a variety of RNases are identified including: RNase M (high concentrations), including RNase genes at least from bovine, murine and rat sources; RNase H, and RNase M are provided including genes from *E. coli.* Further, use of RNases T1 and T2 are taught, including genes from *Aspergillus oryzae.* RNase 4 and human pancreatic RNase are each taught for use with the present invention including their genes. Additionally *Bacillus amyloliquifaciens* BaRNase is provided, and its use is further described at pages 33-35 under Inducible Promoters and Endogenous RNase Secretion Signals.

As to guidance relating to promoters, the specification on the whole demonstrates to those of skill in the art to practice that the inventors were in possession of the invention as claimed. Promoters, of the types described for use with the present invention are well known in the art. The specification teaches multiple means of expressing RNases in various host cells– for example chromosomal integration, or episomal or plasmid expression (application as filed, pages 11-15). Promoters taught include inducible promoters such as phage promoters (recognizing only the phage RNA polymerase), temperature inducible promoters, small molecule inducer activated or repressed promoters, and inhibitor induced promoters. (See application as filed, pages 19-37.)

Working examples are provided from which one of skill in the art would recognize that the inventors were in possession of the invention as reflected in the claims. The examiner alleges that the claims encompass many RNases which are not disclosed by the specification or the prior art. It is difficult to understand exactly what is meant, but it seems some burden is placed on the Applicants for demonstrating possession of RNases that are apparently undiscovered as of yet, but of which the examiner cites from personal knowledge. Applicants assert that their specification establishes that applicants were in possession of the claimed invention. Applicants respectfully request specific citation to art supporting the contention in the Office Action. The Office cannot shift to Applicants the burden to show possession of as yet undiscovered RNases not known in the prior art, and extend this to assert that applicants are not in possession of the invention.

Further, although not every possible embodiment is specifically taught, it need not be. The breadth of the teaching, combined with the working examples and level of skill in the art taken as a whole demonstrate that the Applicants were clearly in possession of the invention as claimed at the time of the filing. The Office Action seems to assert that applicants are required in their specification to put one of skill in the art in possession of the attributes and features of the invention as claimed. Applicants specification is required to demonstrate to one of skill in the art the Applicants were in possession of the invention at the time of filing. Applicants have met this burden. In view of the requirements, one of skill in the art would reasonably conclude that the applicants were in possession of the claimed invention at the time the application was filed. The Applicants respectfully request the rejection be withdrawn.

Claims 4-18 and 37 were also rejected under 35 U.S.C. §112, first paragraph because the specification, while admittedly enabling for a method of preparing RNA-free cellular components in an *E. coli* cell, wherein the cellular component and RNase I or RNase A are produced by said *E. coli* and wherein the RNase I or RNase A are expressed and secreted using an inducible or constitutive promoter, the Office Action alleges the specification is not enabling for a method for preparing RNA-free cellular components wherein any RNase is expressed in any cell or expressed constitutively in the cytoplasm. As discussed above, the instant invention disclosure teaches a wide variety of RNases for use in a wide variety of microbial cells. The RNases, most of which are cloned and for which DNA sequences are readily available to those of skill in the art, range from prokaryotic to eukaryotic, and from microbial to human sources. The Applicants assert that the specification enables a skilled practitioner to practice the invention with any known RNase

The claims are enabled by the specification and it would not require undue experimentation to make and use the invention. The Office Action again cites RNases not known in the prior art. The examiner has the initial burden to establish a reasonable basis to question the enablement provided for the claimed invention.

(*MPEP* 2164.04). The examiner alleges that the claims encompass many RNases which are not disclosed by the specification or the prior art. Again, it is difficult to understand exactly what is meant but it seems the burden is placed on the Applicants to enable hypothetical or unknown RNases, or those which the examiner cites from personal knowledge. Applicants assert that their specification enables the claimed invention. Applicants respectfully request specific citation to art supporting the contention in the Office Action. The Office cannot shift to Applicants the burden for enabling as yet undiscovered RNases not known in the prior art. Applicants respectfully request proper citation to prior art or other source of these allegedly nonenabled species. The guidance as to DNA structures encoding RNases and promoters is more than adequate to enable the claims, as discussed above. The Office Action concedes that one can argue that the prior art discloses the structure of other RNases and that the cloning of the DNA encoding such RNases in several host cells is considered routine in the art, but alleges that the scope of the present claims ecompasses RNases and host cells not disclosed by the specification or the prior art. As discussed above, the Applicants cannot be required to enable unknown RNases or host cells. Further, the Applicants need not provide what is well known in the art and adequate guidance is provided to those of skill.

The Office Action asserts that the teachings of Okorokov et al. (Protein Expression and Purification 6:472-480, 1995) support the idea that expressing RNases in the cytoplasm constitutively would require undue experimentation. Okorokov et al. do not support this contention. Cells are known to natively and constitutively express RNases in the cytoplasm. The statements in the abstract ("Since this degradation of RNA interferes with normal cell functions. . ."), and at page 474 ("This may be attributed to a cytotoxic effect of an intracellularly located RNase or to its sensitivity against host proteases.") are, at most, unsupported speculations. Regardless, the statements of Okorokov et al. do not support a contention that RNases cannot be expressed, but merely that yields may not be optimal. Further, Zhu et al. state "An alternate possibility is that any RNase I within the cell is in an inactive form. It is known that RNase I binds tightly to ribosomes *in vitro* and may do so *in vivo*." Zhu et al., *supra.*

Finally the Office Action alleges that due to lack of relevant examples and information provided, and a lack of information regarding DNA structures encoding RNases, cells and promoters, undue experimentation would be required. Adequate working examples are provided. One of skill in the art, using only the applicants specification would be able to make and use the invention as reflected in the claims.

While some experimentation may be useful in specific circumstances, the quantity of experimentation is not dispositive of the analysis (*MPEP* 2164.04). The key word is "undue," not "experimentation". In re Angstadt, 537 F.2d 498,504 (CCPA 1976). Further, the scope of enablement must only bear a reasonable connection to the scope of the claims. See, e.g., In re Fisher, 427 F.2d 833,839 (CCPA 1970).

As discussed above, adequate disclosure, commensurate with the scope of the claims, is provided. The combined teachings of the specification based on the whole of the evidence show that the specification is indeed enabling for the claimed invention. The specification provides more than adequate guidance to practice the claimed invention. Further, the specification need not be conclusive, but merely convincing to one skilled in the art. (*MPEP* 2164.05) The applicants therefore request that this rejection be withdrawn.

Rejections under 35 U.S.C. §103

Claims 4, 6-7, 9-13 and 15-18 were rejected under 35 U.S.C. §103(a), as allegedly unpatentable over McMaster et al. (Anal. Biochem. 109:47-54, 1980) in view of Okorokov et al. (Protein Expression and Purification 6:472-480, 1995).

The Office Action alleges that it would have been obvious to combine McMaster et al., relating to the production and purification of plasmids, including the addition of exogenous RNase A, with Okorokov et al., relating to the expression of bovine RNase A in E. coli by transforming the cell with a plasmid containing a gene

encoding RNaseA linked to a phoA signal peptide for directing the RNaseA to the periplasmic space and further relating to purifying the RNaseA including an osmotic shock step to form a cell lysate.

In order to assert a proper rejection for obviousness under 35 U.S.C. §103, the following tenets of patent law must be adhered to:

(A) The claimed invention must be considered as a whole;

(B) The references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination;

(C) The references must be viewed without the benefit of impermissible hindsight vision afforded by the claimed invention; and

(D) Reasonable expectation of success is the standard with which obviousness is determined. *Hodosh v. Block Drug Co.*, 786 F.2d 1136 (Fed. Cir. 1986), *MPEP* 2141.

The mere fact the references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. (*MPEP* 2143.01). Here, it would not have been obvious to one of skill in the art to combine McMaster et al. with Okorokov et al. While the purpose of McMaster et al. relates to obtaining purified **DNA**, the purpose of Okorokov et al. relates to obtaining purified **RNase**. There is no language, express or implied, which would lead one of ordinary skill in the art to combine the cell lysate of Okorokov et al. to the DNA purification scheme of McMaster et al.. Furthermore, McMaster et al. report the addition of "purified" RNase with the added requirement of heating the RNaseA at 70C for 30 minutes to destroy DNases. A cell lysate, such as that from Okorokov et al., would be expected to have contaminating DNases, as even the purified commercial RNase used by McMaster et al. was contaminated with DNase. *McMaster et al.*(1990) page 49, column 2.

Therefore, in viewing the references as a whole, from the standpoint of one skilled in the art at the time of the invention without the aid of hindsight, there is no

teaching, or suggestion in either of the prior art references to lead a skilled practitioner to combine or modify the teachings therein to achieve, with a reasonable expectation of success, the results obtained by the applicants in the instant application. There is nothing which separately or in combination would provide motivation for one skilled in the art to combine the teachings or disclosures or to modify them in the required manner, and the examiner fails to provide specific rationale for such motivation other than the bare assertion that the motivation would have been present. (*MPEP* 2142, *Ex parte Skinner* 2 USPQ2d 1788 (BPAI, 1986)). This rejection should be withdrawn.

For the same reasons as stated above, the rejection of Claims 8 and 14 as being unpatentable over McMaster et al. in view of Okorokov et al. and further in view of Zhu et al. (J. Bact. 172:3146-3151, 1990) should be withdrawn. Although, there would have been no motivation to combine the teachings of Zhu et al. relating to constitutive RNase expression to repair a RNase defective strain of bacteria, with those of Okorokov et al. or McMaster et al., even if there were motivation, there would be no expectation of success. A skilled artisan would have been concerned about the same problem that McMaster et al. were concerned with – DNase contamination.

In light of the foregoing arguments and for all the reasons laid out above, the applicant respectfully assert that all claims are novel and non-obvious over the cited references. Accordingly, withdrawal of the rejections under 35 U.S.C. §103 based on McMaster *et al.* and Okorokov *et al.*, and further in view of Zhu *et al.* respectively, is requested.

Summary

In view of the foregoing amendments and remarks, the applicants submit that this application is in condition for allowance and respectfully request early and favorable notification to that effect. If it would expedite prosecution of this application, the Examiner is invited to confer with applicants' undersigned representative.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned **"Version with markings to show changes made."**

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS:

4. (Thrice Amended) A method of preparing a substantially RNA-free cellular component, comprising culturing

(a) cells producing a cellular component; and

(b) cells producing an RNase; wherein the cells producing said RNase are microbial;

lysing said cells producing said cellular component and said cells producing said RNase to produce a combined cell lysate, wherein said cells producing said RNase produce said RNase in an amount sufficient to degrade substantially all of the RNA present in said combined cell lysate, incubating said combined cell lysate to allow said RNase to digest said RNA ~~molecules~~, and isolating said cellular component.

5. (Twice Amended) The method of claim 4, wherein the cells producing the cellular component are the same cells that ~~also~~ produce the RNase.

6. (Thrice Amended) A method of preparing a substantially RNA-free cellular component, comprising culturing cells producing a cellular component and cells producing an RNase, wherein the cells producing said RNase are microbial and wherein the cellular component and the RNase are not produced by the same cells, lysing said cells to produce a combined cell lysate, wherein said cells producing an RNase produce RNase in an amount sufficient to degrade substantially all of the RNA present in said combined cell lysate, incubating said combined cell lysate to allow said RNase to digest said RNA ~~molecules~~, and isolating said cellular component.

7. (Twice Amended) The method of claim 4, wherein said cellular component is selected from the group consisting of ~~a recombinant~~ DNA, ~~a recombinant~~ protein, and ~~a recombinant~~ carbohydrate.

11. (Twice Amended) The method of claim 4, wherein ~~said cell producing~~ expression of said RNase ~~produces said RNase in a~~ is transcriptionally, translationally or post-translationally regulated ~~manner~~.

12. (Amended) The method of claim 11, wherein said RNase ~~produced in said regulated manner~~ is overproduced ~~by said cell producing said RNase~~.

13. (Amended) The method of claim 11, wherein expression of said RNase ~~produced in said regulated manner~~ is ~~inducibly produced by said cell producing said RNase~~ inducible.

14. (Amended) The method of claim 11, wherein expression of said RNase ~~produced in said regulated manner~~ is ~~constitutively produced by said cell producing said RNase~~ constitutive.

15. (Amended) The method of claim 11, wherein said RNase ~~produced in said regulated manner~~ is secreted out of the cytoplasm of the cell producing said RNase.

18. (Amended) The method of claim 11, wherein said RNase ~~being~~ is a non-specific RNase.

37. (Amended) A method of preparing a substantially RNA-free cellular component, comprising culturing cells in a medium, wherein said cells produce said cellular component and RNase~~, and~~; lysing said cells to produce a cell lysate, wherein said cell lysate contains said cellular component and ~~RNase with~~ sufficient RNase activity to degrade substantially all of the RNA ~~molecules~~ present in said cell lysate~~;~~ incubating said cell lysate to allow said RNase to digest said RNA molecules~~;~~ and isolating said cellular component to produce a substantially RNA-free cellular component.